Water Use Efficiency in Plant Biology

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Water Use Efficiency in Plant Biology
Edited by Mark A. Bacon

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Preface

This is the first volume to provide comprehensive coverage of the biology of water use efficiency at molecular, cellular, whole plant and community levels. While several works have included the phenomenon of water use efficiency, and others have concentrated on an agronomic framework, this book represents the first detailed treatment with a clearly biological focus.

The volume sets out the definitions applicable to water use efficiency, the fundamental physiology and biochemistry governing the efficiency of carbon gain *vs* water loss, the environmental regulation of this process and the detailed physiological basis by which the plant exerts control over such efficiency. Chapter 1 offers a general overview of the volume. Chapter 2 provides sound definitions of water use efficiency on which subsequent chapters build. Chapter 3 considers the 'carbon compromise' – the inevitable loss of water incurred at leaf level to gain carbon, and the way in which biochemistry and physiology combine to preserve water use efficiency in an ever-changing environment. Chapter 4 provides in-depth coverage of the environmental control of water use efficiency at leaf level and the role of hydraulic and chemical signalling, which regulate gas exchange, growth and development within the plant. The volume also discusses the role of nutrition in governing water use efficiency, with detailed coverage in chapter 5 of the effects of plant nutritional status on water use efficiency at the single plant and ecosystem levels. Chapters 6 and 7 consider the exploitation of this understanding in agriculture, using agronomic and physiologically-based approaches. This section of the book concludes with a case study demonstrating the application of an understanding of plant water use efficiency in the growing of rice crops in China (chapter 8). The molecular basis of water use efficiency is detailed in chapter 9, which examines our increasing ability to identify water use efficiency traits and phenotypes and to introduce such traits into crop species, using traditional and emerging methodologies. The book concludes with chapter 10, which sets our understanding of the subject, from molecular to community level, in the context of delivering increased water use efficiency in agriculture.

The contributors to this volume represent some of the most prominent researchers in this subject area, who have worked within Europe, Asia, the Americas, Africa and Australia. It is hoped, therefore, that readers throughout the world will be able to relate to the coverage in the context of their native agricultural systems.

The book is aimed at researchers and professionals in plant molecular biology, agriculture, plant developmental biology, plant biotechnology, plant biochemistry and ecology. It will also inform those involved in formulating research and development policy in this topic, in all parts of the world. The book will prove useful for agricultural engineers wishing to gain a better understanding of how potential agronomic and biotechnological advances can be married effectively with agricultural engineering for the efficient use of scarce resources of water.

This book has been made possible by the willingness of contributors to participate in the project and it is a pleasure to acknowledge their professionalism and continued dedication to this area of plant biology. I also wish to thank colleagues and friends who have provided help and advice in the editing of this book and the publication team who have provided support over the last year.

Mark A. Bacon

1 Water use efficiency in plant biology

Mark A. Bacon

1.1 Introduction

This chapter sets the scene for the volume, by reviewing our current understanding of the term 'water use efficiency' in the context of plant biology and the opportunities for exploiting such understanding. A key theme will be the need to integrate our understanding of water use efficiency at the molecular, physiological, biochemical, whole plant and ecosystem levels, because in doing so, we further our empirical understanding and therefore the opportunities for exploitation in modern agriculture.

1.1.1 The global perspective

Global agriculture now accounts for 70 per cent of the amount of water used by humans, with many parts of the world using even more (Figure 1.1). In October 1999, the six billionth person was added to the planet. Rapid global population growth, diminishing agricultural lands due to unsustainable practices, and global climate change mean that, now more than ever before, there is a need to provide technological solutions to achieve sustainable and efficient use of water. Increasing the water use efficiency of crops is one way in which to achieve this (Anderson *et al.*, 1999).

In developed agriculture, losses due to poor nutrition and plant health are greatly reduced (Passioura, 2002) to the extent that crop losses relating to water availability (and failed water use efficiency, in terms of productive yields) continue to exceed those from all other causes (Kramer, 1980). Boyer (1982) successfully illustrated this point by surveying the causes and costs of crop losses in developed agriculture in the United States from 1939 to 1978 (Tables 1.1 and 1.2). By comparison with native populations, Boyer (1982) also established that a large genetic potential for yield can be better realised if breeding programmes develop varieties better adapted for the environments in which they grow – a philosophy that is now firmly embedded in the breeding programmes of the twenty-first century (see below).

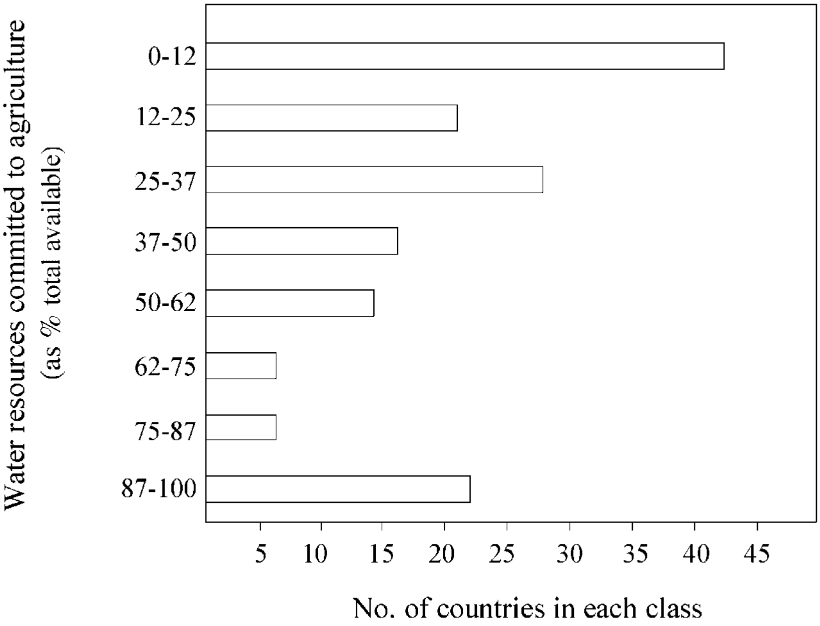

Figure 1.1 Analysis of the extent of water use committed to agriculture (as a percentage of total available water) across 156 countries. Source: United Nations Environment Programme, Global Environmental Outlook Data Portal (http://geodata.grid.unep.ch).

1.1.2 Definition of water use efficiency

Water use efficiency (WUE) does not have a single precise definition. Its definition depends upon the particular context in which it is being discussed, including where the water is in relation to the plant (i.e. inside the plant or in its environment), the time scale over which efficiency is measured (e.g. instantaneous exchange of water vapour for carbon dioxide gas versus biomass accumulation or yield) and the precise measure of efficiency in relation to carbon gain (i.e. carbon dioxide influx, biomass accumulation or economic yield). In most of the current literature, WUE is discussed either in terms of an instantaneous measurement of the efficiency of carbon gain for water loss; or as an integral of such an efficiency over time, (commonly expressed as ratio of water use to biomass accumulation, or harvestable yield). Subsequent chapters will define WUE for the particular context of their discussion.

1.1.3 Historical perspective

According to Stanhill (1986) the first scientific investigation of plant growth and performance in relation to water use was that of Woodward in the

Table 1.1 Area of United States with land subject to environmental limitation (from Boyer, 1982).

Environmental limitation	Area affected (%)
Drought	25.3
Shallowness	19.6
Cold	16.5
Wet	15.7
Alkaline soils	2.9
Saline or no soil	4.5
Other	3.4
None	12.1

Table 1.2 Distribution of insurance payments for crop losses in the USA from 1939–1978. Redrawn from Boyer (1982).

Cause of crop loss	Proportion of payments (per cent)
Drought	40.8
Excess water	16.4
Cold	13.8
Hail	11.3
Wind	7.0
Insects	4.5
Disease	2.7
Flood	2.1
Other	1.5

Philosophical Transactions of the Royal Society, which described the growth increase in spearmint in relation to the expense of water. Lawes (1850) (see Stanhill, 1986) conducted the first set of true experiments to assess arable crop water use, via gravimetric water loss measurements from large containers sown with cereals, pulses and clover and was one of the first to recognise the clear relationship between transpiration and biomass production (see Figure 1.3, p. 8). A landmark in the study of plant water use efficiency came in the early 1900's with the work of Briggs and Shantz (1913) in Akron, Colorado. They determined the transpiration ratio (the ratio of water transpired to dry weight produced – the reciprocal of water use efficiency) for 62 different plant species. Indeed, it would take another 50 years for researchers to realise that the low transpiration ratios recorded for maize, sorghum and millet, could be explained by the existence of C4 photosynthesis (see below). While the validity of the Briggs and Shantz approach was questioned, particularly against emerging meteorological techniques (e.g. Penmon, 1948), reanalysis of the 'Akron series' demonstrated that the analysis was valid and that it was possible to extrapolate the results from container experiments to the field (de Wit, 1958). These studies demonstrated the strict positive relationship between the total amount of water transpired by a crop and its yield (see Figure 1.3, p. 8) by assessing crop yield and water use in response to varying amounts of water (see Kramer and Boyer, (1995) (e.g. Day *et al.*, 1978; Innes and Blackwell, 1981, see also Jones, 1992).

In the latter half of the twentieth century, the discovery that the carbon-isotope fractionation capability of the photosynthetic process, and resultant ratio of stable carbon isotopes within plant tissues could be used to assess both an instantaneous or integrated measure of plant water use efficiency (see Farquhar and Richards, 1984), moved the discussion of water use efficiency firmly within the realms of modern plant science (see below).

1.2 Carbon metabolism and WUE

1.2.1 WUE and the regulation of assimilation

Water use efficiency can be defined as the ratio of CO_2 assimilation into the photosynthetic biochemistry (A) to water lost, via transpiration, through the stomata (T). A and T are regulated by stomatal conductance (g_s) to water and CO_2 and the respective concentration gradients in water vapour (w_i-w_a) and CO_2 (c_i-c_a) between the inside (w_i and c_i, respectively) and outside of the leaf, (w_a and c_a, respectively). Assuming w_i/w_a is independent of c_i/c_a, the so-called 'intrinsic' water use efficiency (W_T) is a negative function of c_i/c_a. Under any particular set of conditions, the driving force for CO_2 uptake will be enhanced by lowering c_i, while the driving force for water loss will remain relatively unchanged, leading to an increase in water use efficiency.

A plant can achieve a lower c_i/c_a ratio (with a concomitant increase in WUE) by decreasing stomatal aperture (lowering c_i by limiting CO_2 diffusion into the leaf interior); increasing photosynthetic capacity for CO_2 (lowering c_i by increasing carboxylation) or more likely, a combination of the two. Indeed, it is commonly observed that stomatal movements can conserve proportionality between c_i and c_a (Wong *et al.*, 1978) with continued debate over if and how photosynthetic capacity, carbon dioxide concentrations and transpiration are sensed and integrated to produce an optimal stomatal aperture (e.g. Cowan, 1982; Farquhar and Sharkey, 1982; Farquhar and Wong, 1984; Jarvis and Davies, 1998).

1.2.2 Photosynthetic biochemistries and WUE

A low mesophyll resistance to CO_2, created by high carboxylation efficiency in the bundle sheath cells of C4 species, ensures a low c_i/c_a and a significant driving force for CO_2 uptake and assimilation. Consequently C4 species can achieve comparable assimilation rates to C3 species at lower stomatal conductances, increasing their water use efficiency. It would also appear that the stomata of C4 species have evolved a reduced direct response to high light, such that a lower internal c_i (and higher WUE) can be maintained under high light conditions, which would usually favour stomatal opening in C3 species (Huxman and Monson, 2003).

With high productivity (under high light and warm temperature environments) there is clear interest in introducing C4 characteristics into C3 crop species, to enhance productivity and WUE. Traditional breeding approaches, involving the hybridisation of C3 and C4 species of *Atriplex*, were less than successful, with independent inheritance of C4 characteristics such as PEP carboxylase and Krantz anatomy (e.g. Björkman *et al.*, 1971). Recombinant DNA technology has enabled the introduction of C4 characteristics into C3 species, including tobacco, potato and rice with some success (Leegood, 2002). This has been achieved primarily via the introduction of enzymatic components of C4 biochemistry rather than attempting to introduce the far more complex leaf structural characteristics of C4 species. Several attempts to introduce C4 characteristics have resulted in clear increases in WUE (Jeanneau *et al.*, 2002a). Transgenic maize lines over-expressing the C4 PEP carboxylase gene resulted in a two-fold increase in PEP carboxylase activity and a 30 per cent increase in the intrinsic water use efficiency (Jeanneau *et al.*, 2002b). For a detailed discussion of molecular engineering of C4 photosynthesis, see Matsuoka *et al.* (2001).

In a similar manner, species exhibiting crassulacean acid metabolism (CAM) have dramatically increased water use efficiencies (10–20 times that of C3 species), by fixing CO_2 during the night when the driving force for water loss is significantly lower. Several species denoted as facultative or inducible CAM exhibit a degree of plasticity in carbon fixing biochemistry, via the use of the C3 pathway when water is sufficient to maximise growth, while switching to CAM metabolism when water supply becomes limiting and evaporative demands are high. Plant families which exhibit such facultative behaviour include the *Crassulaceae, Portulaceae* and *Vitaceae*. In such species, a complex series of gas exchange patterns and stomatal movements can be observed, leading to sustained carbon assimilation (albeit at a much reduced rate) and enhanced water use efficiency as the environment becomes increasingly arid (Cushmann and Borland, 2002). While CAM traits include enhanced WUE, the CAM phenotype is not desirable for introduction into C3 or C4 crop species, due to the low rates of overall biomass accumulation and productivity. However, with increasing atmospheric CO_2 concentrations, and concomitant global warming, the proportion of arid areas of the world is likely to increase, raising the possibility that CAM species may be increasingly cultivated in the future, in order to maintain agricultural productivity.

1.2.3 Isotope discrimination and WUE

The driving force for water loss to the atmosphere from the sub-stomatal cavity is typically 100 times greater than the driving force for CO_2 uptake into the sub-stomatal cavity and photosynthetic biochemistry. In a typical C3 plant, outward fluxes of water vapour may reach 2000–3000 μmol m^{-2} s^{-1}, compared to maximal inward fluxes of CO_2 between 20–30 μmol m^{-2} s^{-1}. There is therefore, an inherent dominance of water loss to carbon gain in all plants. Plants that can generate increased diffusion gradients for CO_2 will therefore enhance their water use efficiency (at constant stomatal conductance).

Two stable isotopes of CO_2 exist and are naturally abundant: $^{13}CO_2$ and $^{12}CO_2$. The $^{13}CO_2$ isotope has a heavier molecular mass, and although its chemical properties are unchanged, its mass dictates a slower molecular speed and diffusion rate into the leaf and a concomitant increased likelihood of collision with other molecules (such as water molecules travelling in the opposite direction under a significant driving force). While the ratio of $^{13}CO_2$ to $^{12}CO_2$ is approximately 1:99 in atmospheric air (O’Leary, 1993), the ratio of $^{13}CO_2$ to $^{12}CO_2$ in plant tissues is reduced even further. Due to its molecular properties, $^{13}CO_2$ is discriminated against along its pathway of diffusion from the atmosphere up to (and including) the point at which it becomes incorporated into organic carbon molecules. As a result, CO_2 arriving at the site of fixation and the carbon in subsequently formed photosynthate is significantly depleted in ^{13}C. For a detailed review of the biochemical basis of carbon isotope fractionation see O’Leary (1993).

The ratio of ^{12}C to ^{13}C can be measured using mass spectroscopy to determine a so-called carbon-isotope signature or level of discrimination against ^{13}C (Δ^{13}C). Graham Farquhar and co-workers have, from the earlier 1980s, pioneered this technique and established that the extent of Δ^{13}C varies according to the partial pressure of CO_2 both inside (c_i) and outside (c_a) leaves and concomitant driving force for CO_2 uptake. A higher c_i/c_a results in a greater Δ^{13}C, while a lower c_i/c_a reduces Δ^{13}C. Consequently, carbon isotope signatures demonstrating a greater Δ^{13}C are diagnostic of a CO_2 fixation environment in which c_i/c_a is relatively high, with signatures exhibiting a lower Δ^{13}C, diagnosing a CO_2 fixation environment with a relatively low c_i/c_a. A low c_i/c_a is diagnostic of a higher WUE, due to the relatively greater driving force for CO_2 uptake generated by a low c_i/c_a, (Figure 1.2). The relationship between Δ^{13}C (and c_i/c_a), is a function of photosynthetic capacity of the mesophyll and stomatal conductance, with Δ^{13}C reflecting the indicative set point (or optimum) of these two parameters in any particular instance. A healthy C3 plant will have a c_i/c_a ratio of about 0.7 (Farquhar *et al.*, 1989).

In C4 plants, the primary carboxylating enzyme, phosphenol-pyruvate (PEP) carboxylase has a significantly reduced ability to discriminate against

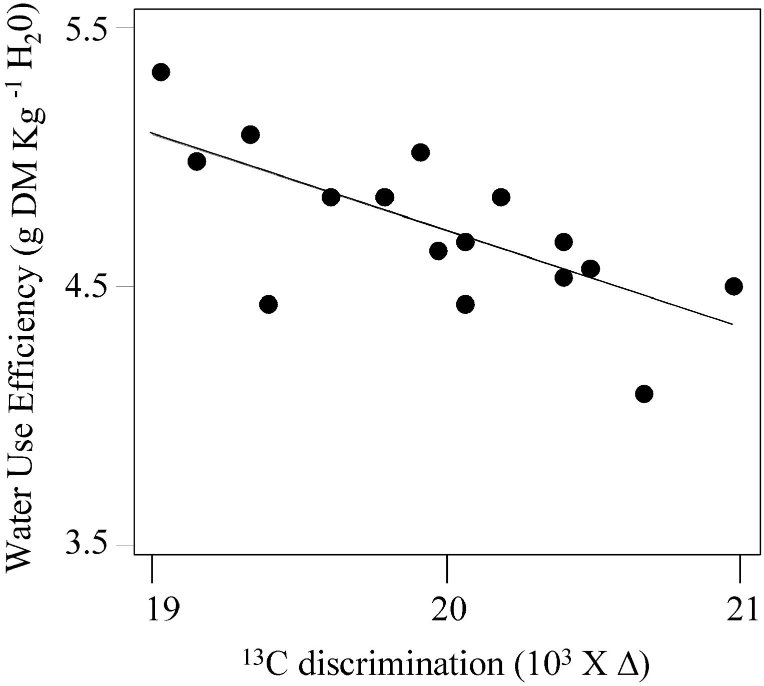

Figure 1.2 Illustrative relationship between $\Delta^{13}C$ and WUE in differing genotypes grown under well-watered conditions (adapted from Condon *et al.*, 1990).

$^{13}CO_2$. Coupled with the ability of C4 photosynthesis to establish a significantly greater driving force for CO_2 uptake (when compared to C3 species), $\Delta^{13}C$ in C4 plant tissue is significantly lower.

Farquhar and co-workers have demonstrated the value of this approach in several crops, by determining the carbon isotope signature and relating a low level of discrimination with enhanced WUE (e.g. Condon *et al.*, 1987; Hubrick *et al.*, 1986; Hubrick and Farquhar, 1989). Importantly, this relationship holds whether measured instantaneously via gas exchange analysis (e.g. Evans *et al.*, 1986) or from plant material integrating carbon fixation over the lifetime of the tissue (e.g. Farquhar and Richards, 1984). But do genotypes expressing enhanced WUE deliver enhanced productivity (i.e. WUE expressed on a yield basis)? This question forms the basis of one of the case studies highlighting how an understanding of WUE in plant biology can deliver significant agricultural benefits (see below).

While the fundamental understanding of $^{13}CO_2$ discrimination in plants has delivered tangible benefits via new cereal varieties with higher water use efficiencies (see below) using $\Delta^{13}C$ alone does not give any information on

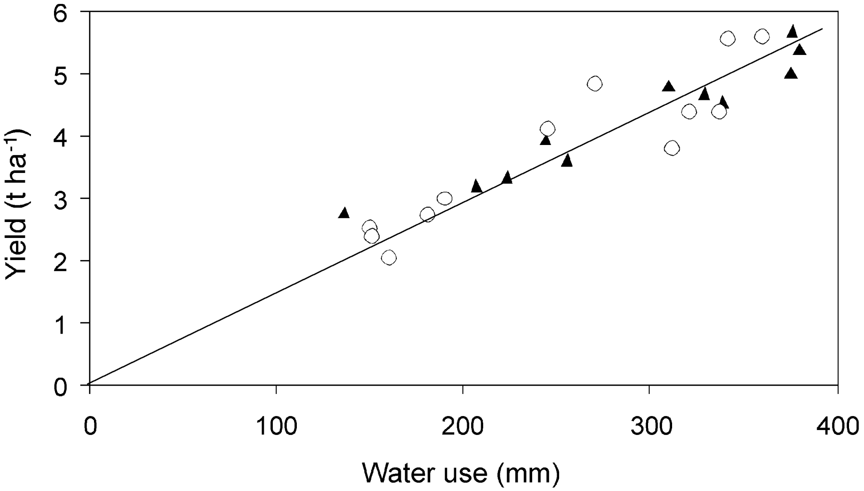

Figure 1.3 Grain yield as a function of water use under a range of irrigation treatments for barley in 1976 (○) and wheat in 1970 (▲) in south-eastern England (data from Day *et al.*, 1978; Innes and Blackwell, 1981). The slope of the line through the origin indicates the WUE for any treatment.

the relative contribution of photosynthetic or stomatal control of c_i and thus the contribution of stomatal regulation in determining water use efficiency. If high WUE is conferred by low stomatal conductances, productivity under well-watered environments may not be as great as that from a genotype with a lower WUE, a consequence of the strict relationship between transpiration and productivity (Figure 1.3). The use of stable isotopes of oxygen may however, be beginning to provide an ability to assess whether highly WUE genotypes (as identified by $\Delta^{13}C$) are also those with high levels of transpiration (e.g. Barbour *et al.*, 2000).

When water evaporates from the sub-stomatal cavity, the leaf becomes enriched in the O^{18} isotope of oxygen, due to the enhanced evaporation of H_2O^{16} molecules, relative to heavier H_2O^{18} molecules (a consequence of differences in their molecular mass). At a stable vapour pressure deficit, stomatal opening will cause a drop in internal partial pressure of H_2O, and thus increase the driving force for water loss. Under such conditions, enrichment at the site of evaporation is reduced (in an analogous manner to $\Delta^{13}C$, in which an enhanced driving force for CO_2 uptake increases ^{13}C enrichment of tissues). However, total leaf tissue H_2O appears less enriched than that at the site of evaporation, and this disparity increases with transpiration (Flanagan *et al.*, 1994). The reason for this increasing discrepancy is thought to result from diffusion of enriched water (specifically H_2O^{18}) close to the site of evaporation into the leaf away from the sites of evaporation, being opposed by the flux of water from the xylem into the leaf via transpiration flow (the so-called Péclet effect). Increased stomatal conductances will increase the transpirational flux, increasingly opposing the diffusion of H_2O^{18} into the leaf and thus reducing the overall enrichment of bulk leaf tissue. The Péclet effect therefore has the same effect on bulk leaf enrichment as that caused by the decreased fractionation of H_2O^{16} and H_2O^{18} under enhanced evaporative demand. The overall effect is that increased stomatal conductance results in a decrease in H_2O^{18} enrichment. The dominance of both the Péclet effect and fractionation at the sites of evaporation changes depending on climatic conditions such as relative humidity and/or temperature.

The oxygen isotope signature is ultimately stored in leaf carbohydrates, via the molecular exchange of oxygen between water and carbonyl groups within leaf carbohydrates, although the signature is dampened by the fact that the exchange of oxygen molecules between water and carbonyl groups exhibits reduced discrimination against O^{18}. It is possible to model and test the relationship between O^{18} discrimination in bulk leaf tissue and stomatal conductance and the relationships between C^{13} and O^{18} discrimination (Barbour *et al.*, 2000). It can therefore be predicted that as stomata close, ^{13}C discrimination would decrease and ^{18}O discrimination increase and several authors do report this negative relationship (e.g. Saurer *et al.*, 1997). As with measurement of carbon ratios in bulk tissue, oxygen ratios can therefore provide an integrated measure of transpiration, with low levels of enrichment indicative of high rates of transpiration throughout the lifetime of the tissue sampled. The clear relationship between seasonal transpiration and productivity (see above), therefore makes screening of genotypes with low levels of ^{18}O enrichment in tandem with low ^{13}C enrichment, a powerful tool to deliver high yielding, highly water use efficient crop varieties (see below).

1.3 Stomata and WUE

From the discussion above, it is clear that the regulation of stomatal aperture is central to the water use efficiency of plants. Light, temperature, humidity and carbon dioxide concentrations will all act in some way either directly or indirectly on the stomatal aperture, together with internal circadian rhythms, leaf water status and xylem borne signals (e.g. cytokinins, abscisic acid, etc.). At any point in time all of these signals must in some way be integrated to deliver a particular aperture under a particular set of environmental conditions. While work at the cellular and molecular level is beginning to illustrate how such integration may occur (e.g. Webb and Hetherington, 1997) uncertainty on how (or if) stomatal guard cells actually sense some of these environmental signals (particularly CO_2), has remained a source of active debate (e.g. Ball and Berry, 1982; Raschke, 1986; Mansfield *et al.*, 1990; Kearns and Assmann, 1993; Jarvis and Davies, 1998) particularly in relation to the indirect role of mesophyll photosynthesis in controlling stomatal conductance (see Farquhar and Wong, 1984).

Irrespective of these uncertainties it seems intuitive (and clearly advantageous) that stomatal guard cells continually integrate environmental signals from both the aerial environment and the soil, to generate a stomatal aperture, which optimises water loss and carbon dioxide gain under a given set of environmental conditions. Optimisation theory of stomatal aperture (see Cowan, 1982; Farquhar and Sharkey, 1982) predicts that stomatal aperture varies during the day to ensure minimum water loss for maximum carbon gain. As such, while any increase in stomatal conductance generates a proportionally greater increase in transpiration than assimilation, optimisation theory predicts that such variation will keep the ratio of such changes in transpiration and assimilation rate constant, thus preserving intrinsic WUE (see Jones, 1992). Cowan (1982) provides a detailed discussion of optimisation theory, illustrated by considerations of optimisation in relation to diurnal fluctuations in leaf microenvironment and soil water supply, over time periods of relevance to the overall WUE of a plant throughout its development.

While the role of stomata in governing the driving force for CO_2 influx is well appreciated, stomata do have a limited ability to change the driving force for water loss via transpirational leaf cooling. An enhanced ability to achieve leaf cooling (in the absence of any changes in stomatal function), will reduce the internal partial pressure of water vapour and the concomitant driving force for water loss via evaporation. While only a minor trait conferring a theoretical degree of water use efficiency, novel thermal imaging technology can now detect such subtle differences in leaf temperature in both laboratory and field environments (Jones *et al.*, 2003)

As will be discussed in Chapter 2, it is important to recognise that changes in stomatal conductance (and resultant changes in the efficiency of water use) may not necessarily scale to the crop and ecosystem level, due to a series of crop level factors (i.e. canopy boundary layer conductance and temperature due to latent heat of evaporation from the crop surface) which reduce and ‘decouple’ the stomatal influence on transpiration (see Jones, 1993 and Chapter 2). These factors are also central to understanding the discrepancies often observed between predicated and realised increases in water use efficiency which may be achieved as global atmospheric CO_2 concentrations rise (see e.g. Polley, 2002 and Chapter 3).

1.4 Leaf growth and WUE

While changes in leaf size may also change the CO_2 and H_2O fluxes into and out of the leaf, due to modification to the leaf boundary layer, differences in leaf thickness will also have a significant effect on water use efficiency, with thinner leaves (with a lower ratio of internal volume to leaf surface area) predicted to exhibit lower water use efficiencies than comparable thicker leaves (Stanhill, 1986).

It has also been suggested that rapid leaf development in annuals contributes to the efficiency of water use in the soil. By establishing a high specific leaf area quickly, evaporation of water from the soil is minimised and 'stored' close to the plant, such that it can be drawn upon later in development when water may become limiting (e.g. López-Castañeda *et al.*, 1996). This trait has recently been exploited with some success to deliver more water use efficient and high yielding wheat lines, (see Asseng *et al.*, 2003). As well as the potential direct effects on soil water evaporation, Blum (1996) also suggests that such an adaptation will also minimise the potential for surface roots to come into contact with drying soil, and reduce the likelihood of initiating root-borne signals inhibitory to stomatal conductance. Conversely however, rapid leaf area development may actually prevent soil interception of precipitation, enhancing the rate at which soil water is depleted (Blum, 1996). Clearly this will depend upon the rainfall patterns in a particular environment. The development of a WUE phenotype, well adapted to the environment, is discussed in Chapter 10.

Increasing the photosynthetic capacity of the mesophyll will enhance water use efficiency. However, increased photosynthetic capacity is often associated with a decrease in leaf size (Bhagsari and Brown, 1986), reducing whole plant transpiration and light interception, such that WUE on a plant biomass basis, over time, may actually decrease. The decline in leaf area is likely to occur if any increase in photosynthetic capacity results from an increase in the concentration of enzymes associated with the photosynthetic biochemistry. Under such circumstances, enhanced photosynthetic capacity and limited nitrogen resources are optimised, such that specific leaf area declines (i.e. there is an increase in the dry matter content of leaves on a leaf area basis).

When soil water availability is limited, leaf expansion rates are commonly observed to decline (see Bacon, 1999) in line with transpiration. The innate relationship between transpiration and yield (Figure 1.2) would suggest that this leads to an overall decline in biomass production (and yield productivity of commercial crops). In a majority of cases this is certainly the case. However, under some circumstances, yield and WUE can be sustained or even enhanced even though there is a decline in biomass accumulation at the whole plant level. This would certainly appear to be the case in some cultivated species exhibiting excessive leaf area development when irrigated (e.g. *Vitis* *vinifera*), which can withstand declines in biomass production (particularly vegetative leaf biomass) due to decreased water availability, without any negative effects on yield (see Chapter 5).

1.5 Roots, hydraulic conductivity and WUE

Deep and expansive root systems are an apparent strategy to ensure maximal water use efficiency in terms of water extraction from the soil, particularly when water availability in soils may decline. An ability to continue to develop deep and advantageous roots when soil conditions become increasingly limiting to root growth will enhance the ability of plants to extract available water efficiently. It is a commonly observed phenomenon that as soil water availability declines, the ratio of roots to shoots typically increases. There are very few data that suggest that root growth can actually be increased by soil drying. Those that do (e.g. Sharp and Davies, 1979), attribute such effects to a stress of particular magnitude which results in increased availability of assimilates to roots, as shoot growth is limited by water deficit in the absence of any effect on carbon gain. More recently, however, Mingo (2003) has reported that under particular circumstances, root growth can be stimulated when roots are rehydrated after a drying episode, relative to roots in moist soil.

Water use efficiency in terms of ability to sustain water extraction from the soil, becomes increasingly difficult as the soil dries. Soil drying places a number of different constraints on the growth and functioning of roots and most of these are poorly understood due to the highly heterogeneous nature of the rooting environment, the delicate nature of the relationship between roots and soil structure and the difficulty of investigating root growth and functioning without disrupting this relationship. One of the common responses to soil drying is that roots show enhanced geo-tropism (e.g. Sharp and Davies, 1985). An increased rooting depth can significantly increase water uptake by root systems even when relatively few roots are involved. The adaptive significance of sustaining root growth (even if at a reduced rate) is only clear, however, if plants are competing in natural communities for different soil water resources. There would appear to be nothing to be gained by plants in a monoculture investing increased carbohydrate into deeper rooting when all plants in the stand are competing for the same reserves of soil water (Bacon *et al.*, 2003). As soil water potentials fall, in substrates with a low mechanical impedance (i.e. roots can penetrate the substrate easily), roots have been observed to thin, an adaptation presumably to commit limited carbohydrate supply to extension growth and allow plants to explore deeper water reserves (Sharp *et al.*, 1988). However, in most soils, decreasing water potentials are commonly associated with increased mechanical impedance, such that roots

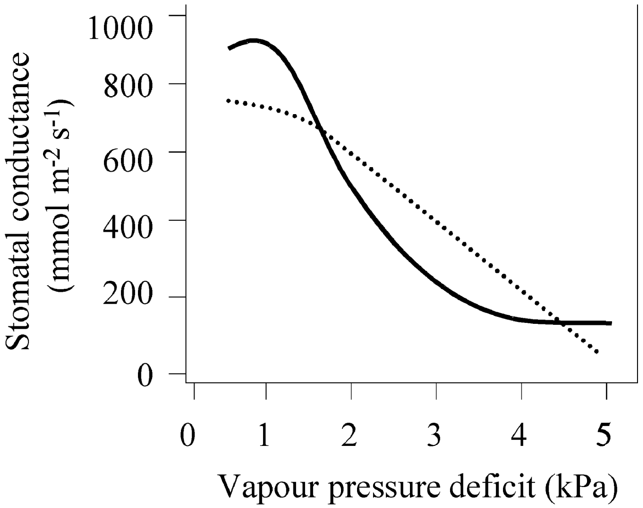

Figure 1.4 Two illustrative responses of stomatal conductance to increasing vapour pressure deficit (adapted from a figure in Atwell *et al.* (1999) which used unpublished data of D. Eamus).

have been shown to swell as soil dries, particularly behind the root apex (Spollen *et al.*, 2000). The prevalence of this phenomenon may allow roots to continue to penetrate the soil as its mechanical impedance increases on drying which may be related to a capacity to generate high turgors in root tips (see Richards and Greacen, 1986; Atwell and Newsome, 1990). Roots of many plants in compacted soils are restricted to cracks in the soil structure. As a result, roots will often grow down these fissures causing substantial localised drying, even when the water content of the bulk soil is still substantial.

Overall transpirational flux through a plant is determined by the characteristics of a plant's hydraulic architecture. Any change in these characteristics could lead to an alteration in the response of open stomata to transpiration rate (see Figure 1.4) and an effect on water use efficiency. This may be particularly important when considering water use efficiency over a prolonged period of time during development. Narrower xylem vessels in roots will result in an overall increase in the hydraulic resistance to water flow throughout the plant (Richards *et al.*, 2002). Consequently, efficiencies in water use may be gained by restricting water uptake early in the development of the plant, to ensure sufficient soil water is available during the reproductive stage. This trait has been confirmed as advantageous in breeding programmes for xylem diameter in wheat (Richards and Passioura, 1989) in which introduction of decreased xylem diameter as a selectable trait increased yields by about 7 per cent.

Sperry and co-workers have recently developed a hydraulic model of water transport through the soil-plant-atmosphere continuum in relation to the component driving forces (and the encountered resistances) to predict transpirational rates, on the basis that plants have evolved hydraulic stomatal optimisation mechanisms to ensure water loss does not exceed uptake by the roots (see Sperry *et al.*, 2002). However, the growing evidence of the root-sourced signals emanating from the root eliciting control of transpiration, does question such a purely hydraulic model. It is becoming increasingly clear that root-sourced signals appear to play a key role in regulating stomatal aperture in response to soil water availability, such that these signals may provide the means by which water supply would appear to regulate water loss.

When soil water availability falls below a certain level, root water potentials and turgors can reach very low values and stimulate the synthesis of several plant growth regulators including abscisic acid (ABA) (Wright, 1977). It is now well established that the production and export from roots of ABA can be related to soil water status and may act as a suitable candidate messenger to ensure the demand for water from the plant is closely controlled by water supply from the roots. The exact roles of hydraulic and chemical regulation of transpiration water loss will continue to attract serious debate and be the subject of future research, with potentially significant impact on understanding (and exploitation of) the physiological basis to water use efficiency. Wilkinson (Chapter 4) extends the discussion of chemical messengers and environmental perturbations which generate them, in relation to the control of plant gas exchange and growth, particularly when water availability declines.

Very interestingly, Holbrook and co-workers have shown that the concentration of potassium ions moving through the xylem can influence the hydraulic conductivity of the transport pathway, perhaps by affecting the nature of the pit membranes within xylem vessels (Zwieniecki *et al.*, 2001), such that a root-sourced chemical signal can influence the properties of the water transport pathways through the root and therefore influence the hydraulic signalling between the roots and shoots. This interesting observation marries exclusively hydraulic- and chemical-based signalling hypotheses together, in a way which brings new knowledge to the understanding of how transpirational water flux may be regulated – with profound effects on the overall water use efficiency of the plant.

Of some increasing interest is the functional significance of aquaporins, hydrophobic proteins which facilitate the movement of water across plasma membranes (Tyerman *et al.*, 2002). It is estimated that plant aquaporins transport the highest total mass of any substance through a plant, when the volume of transpirational water loss is considered, illustrated by the significant reduction in transpirational flux induced by aquaporin inhibitors (see Tyerman *et al.*, 1999). While not contributing directly to the apoplastic flow of water, aquaporins will regulate the cellular flow of water, which becomes increasingly important as water availability declines. It is currently difficult to predict however, what would be the exact involvement of aquaporins in regulating plant water use efficiency. One may envisage that when stomata are closed (as soil water availability declines), decreased aquaporin activity may prevent loss of water to the soil or increased water flow into cells with an enhanced ability to store water (Tyerman *et al.*, 1999). The ability of aquaporins to potentially regulate the turgor of cells in roots and leaves would offer the potential to place the hydraulic conductivity of a plant under metabolic control. In terms of water use efficiency, this may have profound consequences, such that significant xylem tensions, which develop as soil water availability declines, could be partially relieved. Under such circumstances, if stomatal closure in response to soil drying can, in part, be attributable to prevention of xylem cavitation (see Jones, 1993), it may be suggested that such a relief of xylem tension would permit an enhanced level of stomatal conductance and continued fixation of carbon, as water availability declines.

1.6 Uncovering the genetic basis to WUE

An early observation by Martin and Thorstenson (1988) demonstrated that crossing a variety of tomato (*Lycopersicon esculentum* L.) exhibiting a low water use efficiency, with a wild relative (*Lycopersicon pennellii* L.) with high water use efficiency, produced hybrids exhibiting intermediate WUE (Figure 1.5). Differences in WUE between the parental lines and the F1 hybrids were correlated with restriction fragment maps of the tomato DNA and shown to associate closely with three loci within the tomato genome (Martin *et al.,* 1989).

While these initial observations suggest a relatively small number of genes implicated in the genotypic variation, it is readily conceivable that many of the traits conferring WUE will be determined by multiple genes, making breeding programmes for WUE potentially complex. Recently Rebetzke *et al.*, (2003) have explored the inheritability of stomatal conductance traits in wheat cultivars, revealing complex additive and non-additive effects important in the expressed conductance phenotype.

The availability of linkage maps based on molecular markers facilitates the genetic analysis to complex physiological traits such as water use efficiency. Methodologies such as quantitative trait loci (QTL) analysis permit the assignment of variation evident between either environmental or genetic factors to an estimate of the number and location of genetic loci controlling a trait of interest, together with the relative contribution of component traits in an overall phenotype and the development of linked molecular markers. Such markers can be used as selectable markers in conventional breeding programmes and offer the possibility of identifying (and modifying) particular

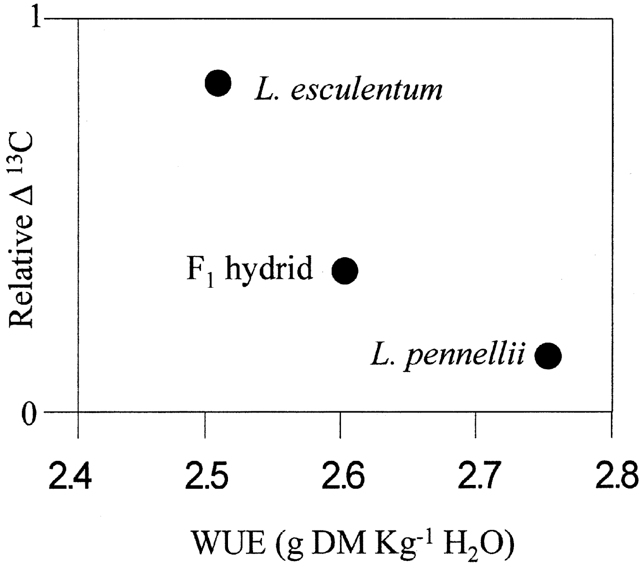

Figure 1.5 The heritability of carbon isotope discrimination and WUE. The F1 progeny of a cross between a domesticated tomato (*Lycopersicon esculentum*) with low WUE (and high $\Delta^{13}C$) and wild tomato relative (*Lycopersicon pennelli*) with high water use efficiency (and low $\Delta^{13}C$), show intermediate behaviour (adapted from a figure in Kramer and Boyer (1995) which used data from Martin and Thorstenson (1988)).

genes generating the trait of interest, by assessing DNA sequence homology of candidate genes with that of sequence close to identified genetic loci or sequencing and identification of new genes at the locus of interest.

As discussed earlier, the development of an efficient root system will increase soil water use efficiency. Via linkage to a molecular marker Champoux *et al.* (1995) have shown that the QTL for root characteristics in rice mapped close to those appearing to regulate drought resistance via enhanced soil water use efficiency. Similar traits in maize have also been reported (Lebreton *et al.*, 1995). With the clear interest in the value of carbon and oxygen isotope discrimination in predicting WUE, several attempts have also been made to establish the loci determining such discrimination. Accordingly, using wheat/barley addition lines (in which individual chromosomes of barley are isolated in a wheat background), Handley *et al.* (1994) investigated the effects of each of the seven barley chromosomes in conferring reduced ability to discriminate against $^{13}CO_2$. Only chromosome 4 was found to have any effect on isotope discrimination. In a similar manner, QTLs for reduced carbon isotope discrimination (and inferred water use efficiency properties) have been identified on chromosomes 1BS and 6BS in the genome of hexaploid wheat (Quarrie *et al.*, 1999). Physiological traits